Antibacterial agents

Antibacterial agent is a synthetic or naturally occurring agent which can kill (Bactericidal) or inhibit the growth (Bacteriostatic) of bacterial cells.

Antibiotic agent is an antibacterial agent derived from a natural source.

Antibiotics: are classified into

- β-Lactam Antibiotics
  - ✓ Classical β-Lactam Antibiotics : Penicillins and Cephalosporins.
  - ✓ Non-classical β-Lactam Antibiotics :
  - ✓ Carbapenems, Monobactams and β-Lactamase inhibitors.
- Non β -lactam Antibiotics
  - ✓ Tetracyclins, Marcolides, Aminoglycosides, Chloramphenicol, …etc
- Synthetic chemotherapeutic Agents.
  - ✓ Sulfonamides
  - ✓ Antimycobactrial drugs
  - ✓ Quinolones
  - ✓ Antiparasitic Drugs
  - ✓ Antifungal Agents
  - ✓ Antiviral Agents
  - ✓ Anticancer Agents

The success of antibacterial agents results from their selective action against bacterial cells rather than animal cells due to the difference in their structure and biosynthetic pathways.

Animal cell	Bacterial cell
✓ has a defined nucleus	✓ does not have a defined nucleus
✓ contain a variety of structures called organelles (e.g. mitochondria, etc.),	✓ relatively simple
✓ It can acquire intact essential vitamins from food	✓ Synthesize essential vitamins, so having enzymes catalyzing these reactions.
✓ has only cell membrane	✓ has a cell membrane and a cell wall

Mechanisms of antibacterial action.

There are five main mechanisms by which antibacterial agents act:

1. Inhibition of cell metabolism (antimetabolites): sulphonamides.

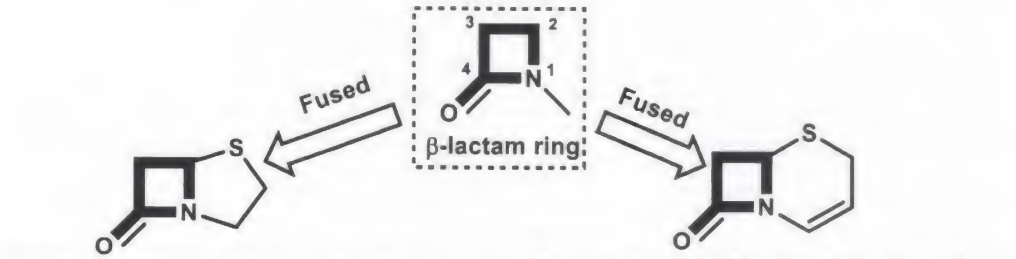
2. Inhibition of bacterial cell wall synthesis (leads to bacterial cell lysis and death):- penicillins, cephalosporins, and glycopeptides
3. Disruption (inhibition) of protein synthesis (so essential proteins and enzymes can no longer be made): rifamycins, aminoglycosides, tetracyclines, and chloramphenicol.
4. Inhibition of nucleic acid transcription and replication prevents cell division and/or the synthesis of essential proteins: Quinolones.
5. Interactions with the plasma membrane (so affect membrane permeability which fatal results for the cell): Polymyxins.

β-lactam antibiotics

β-lactam antibiotics are antibiotics which contain the β-lactam ring (4-membered cyclic ring, azetidinone: 1-azacyclobutan-4-one).

β-lactam antibiotics are classified into:

- ✓ Classical types: Penicillins and Cephalosporins.
- ✓ Non-classical β-Lactams: Carbapenams, Monobactams and other related antibiotics.

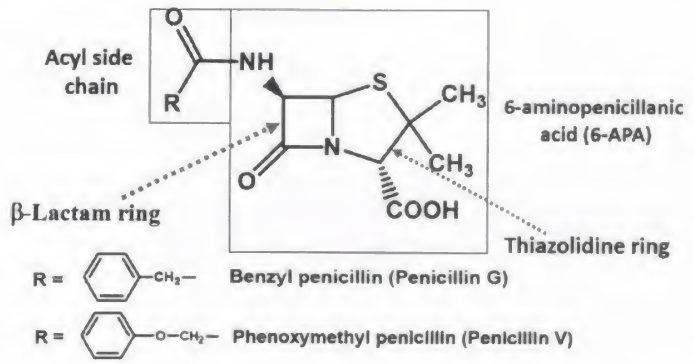


5-membered thiazolidine ring:
Penam
(Penicillin)

6-membered dihydrothiazine ring;
Cephem
(Cephalosporin)

1- Penicillins

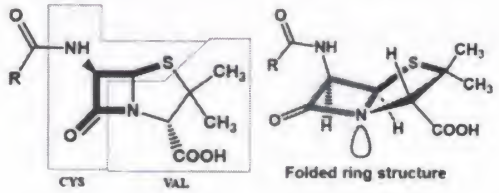
- ❑ Penicillin contains highly unstable bicyclic ring system consisting of:
  - ✓ 4-membered β-lactam ring
  - ✓ Fused to a 5-membered thiazolidine ring.



The skeleton of the molecule suggests that it is derived from the amino acids:

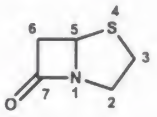
- ✓ Cysteine
- ✓ Valine

The overall shape of the molecule is like a half-open book.



Nomenclature of Penicillin

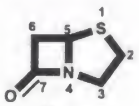
- **Chemical abstract system:** - As bicyclic system



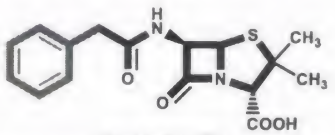
4-Thia-1-azabicyclo[3.2.0]heptane-7-one

Benzylpenicillin
3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

- **Penam derivatives**
  - ✓ It is the name given to unsubstituted bicyclic lactam.

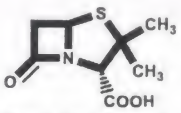


Penam

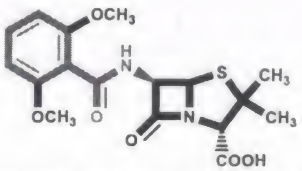


Benzylpenicillin
2,2-dimethyl-6-[(phenylacetamido]penam-3-carboxylic acid

- **Penicillanic acid derivatives**

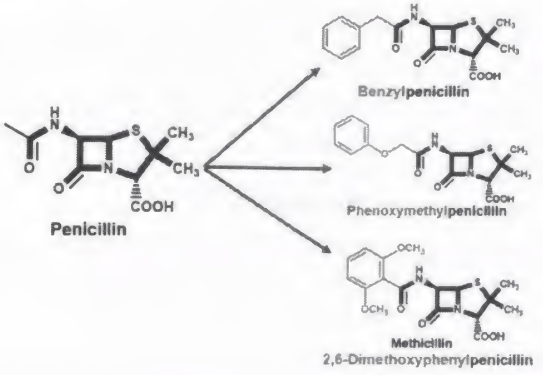


Penicillanic acid



6-(Dimethoxybenzamido)penicillanic acid

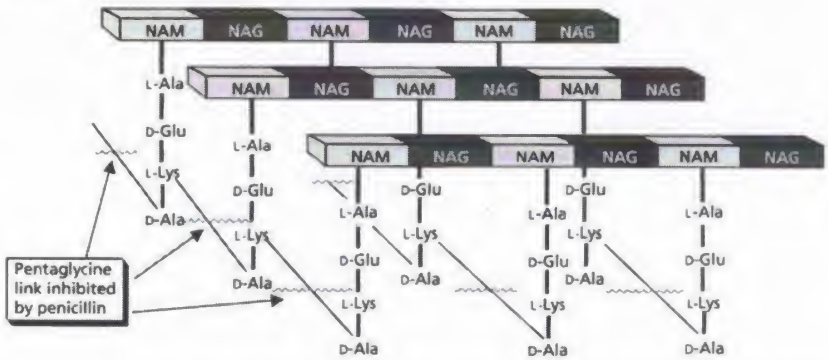
- **Penicillin derivatives:**



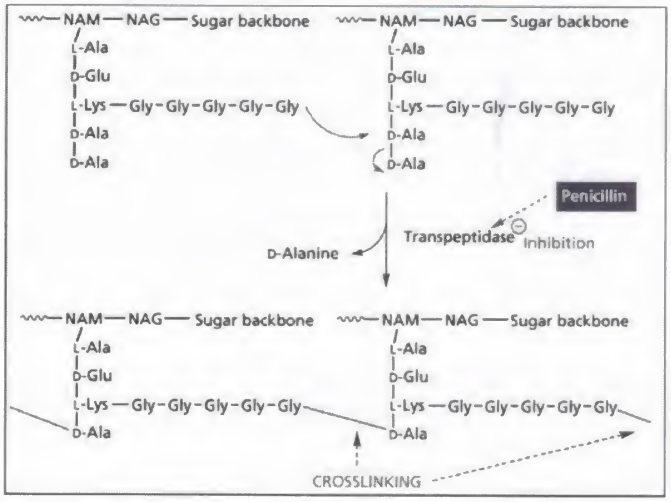
Mechanism of action of Penicillin

Animal cells do not have a cell wall, making it the perfect target for antibacterial agents such as penicillins. The bacterial cell wall is a peptidoglycan structure: it is made up of peptide and sugar units. The structure of the wall consists of a parallel series of sugar backbones containing two types of sugar: N -acetylmuramic acid (NAM) and N - acetylglucosamine (NAG)

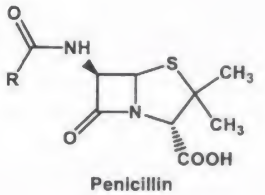
Peptide chains* are bound to the NAM sugars. The peptide chains are linked together (by the displacement of D-alanine from one chain by glycine in another) by transpeptidase enzyme (Penicillin binding proteins; PBPs).



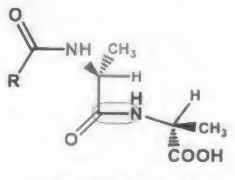
Inhibition of transpeptidase leads to a weakened cell wall . Cells swell due to water entering the cell, then burst (lysis).



The penicillins and the other β-lactam antibiotics have a structure that closely resembles that of acylated D-alanyl-D-alanine.



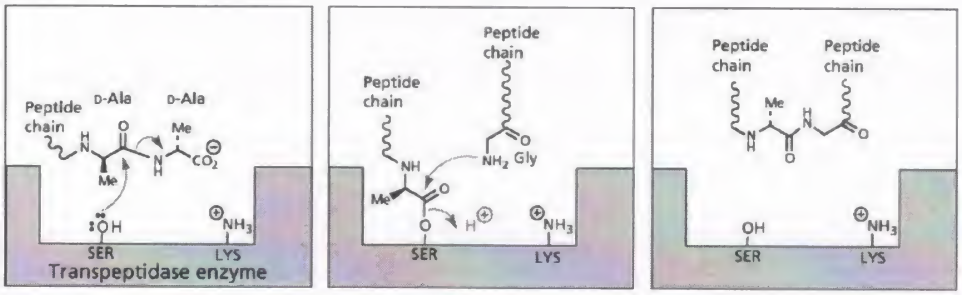
Penicillin



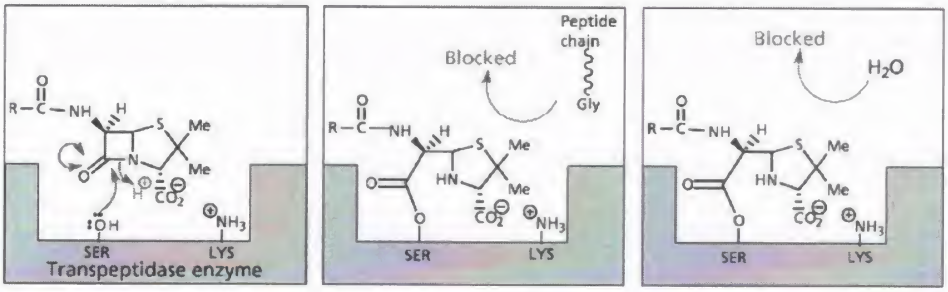
Acyl-D-Ala-D-Ala

The enzyme mistakenly accepts the penicillin as though it were its normal substrate. Once bound, penicillin is subjected to nucleophilic attack by serine moiety of the transpeptidase enzyme (PBPs).

Normal Mechanism



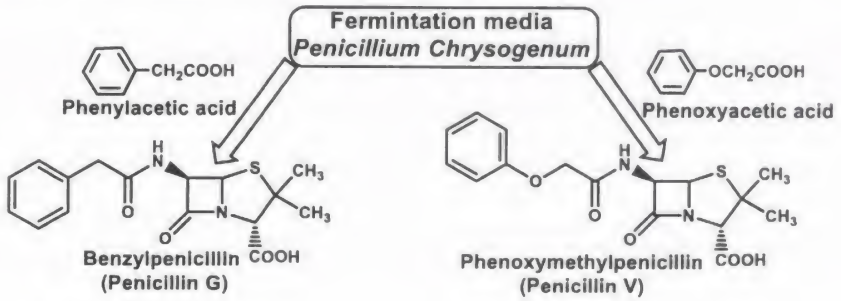
Mechanism inhibited by penicillin



Sources of Penicillins

1- Natural Penicillins

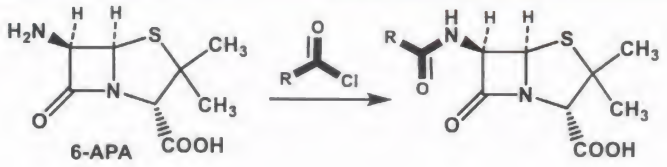
- Fermentation: varying the fermentation conditions
  - ✓ Adding different carboxylic acids* to the fermentation medium resulted in penicillins with different acyl side chains e.g. Benzylpenicillin (penicillin G) and Phenoxymethylpenicillin (Penicillin V)



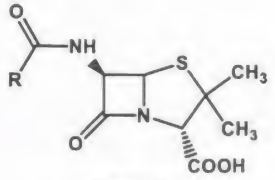
2- Semi-Synthetic Penicillins

Acylation of 6-Aminopenicillanic acid (6-APA)

The isolated biosynthetic intermediate 6-APA from *Penicillium chrysogenum* grown in a fermentation medium was treated with a range of acid chlorides.



SAR of penicillins



- ✓ The strained β-lactam ring and the bicyclic system is essential.
- ✓ This confers further strain on the β-lactam ring: - The greater the strain, the greater the activity, but the greater the instability of the molecule
- ✓ The free carboxylic acid is essential.
- ✓ This is usually ionized and penicillins are administered as sodium or potassium salts.
- ✓ The carboxylate ion binds to the charged nitrogen of a lysine residue in the binding site.
- ✓ The acylamino side chain is essential.
- ✓ The stereochemistry of the bicyclic ring with respect to the acylamino side chain is important.
- ✓ Sulphur is usual but not essential.

Any variations are restricted to the acylamino side chain (R)

Resistance to penicillin

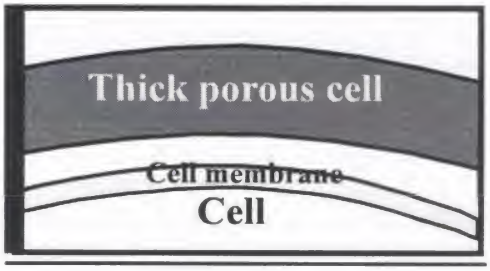
Bacterial strains vary in their susceptibility to penicillin. There are several reasons for this varied susceptibility.

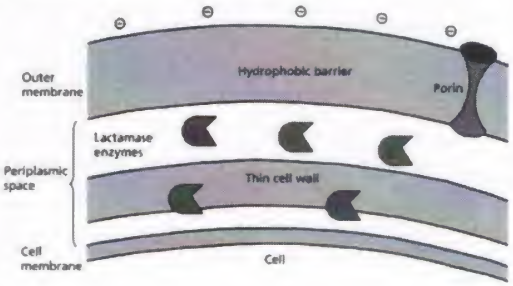
1. Physical barriers.
2. Presence of β-lactamase enzymes.
3. High levels of transpeptidase enzyme (PBPs) produced.
4. Affinity of transpeptidase enzyme to penicillin.
5. Transport back across the outer membrane of Gram-negative bacteria (Efflux pumps).
6. PBP Mutations and genetic transfers.

1- Physical barriers

- ✓ Penicillin has to pass through the cell walls of both Gram-positive and Gram-negative bacteria to reach the outer surface of the bacterial cell membrane to inhibit the transpeptidase enzyme.
- ✓ The cell wall is highly porous, so small molecules as penicillin can move easily through it.

✓ In Gram-positive bacteria, there is no barrier preventing penicillin reaching the cell membrane so penicillin G has good activity against these organisms.





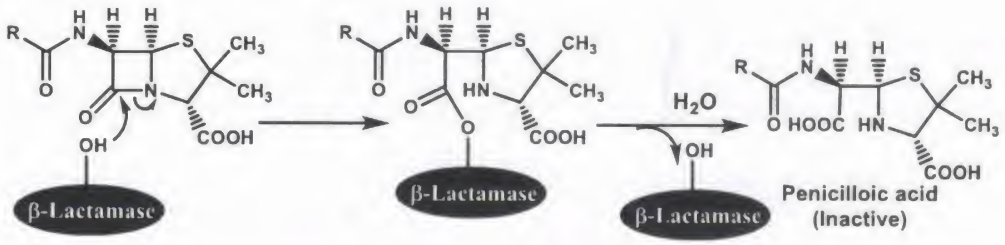
However, Gram-negative bacteria have an outer lipopolysaccharide membrane surrounding the cell wall which is impervious to water and polar molecules, such as penicillin. So, Gram-negative bacteria are generally resistant to penicillin.

The outer membrane has a protein structures called porins which act as pores through which water and essential nutrients can pass to reach the cell.

Small drugs can also pass through porins, but whether they do or not depends on the characteristics of the penicillin (i.e. its size, structure, and charge), HOW?

2- Presence of β-lactamase enzymes

β-lactamases are enzymes which have mutated from transpeptidases. They hydrolyze (open up) the β-lactam ring of benzylpenicillin.



- Some Gram-positive bacterial strains are resistant to penicillin as they can release β-lactamase into the surrounding environment so penicillin is inactivated before reaching the cell membrane. The enzyme eventually dissipates through the cell wall and is lost, so the bacterium has to keep generating the enzyme to maintain its protection.
- All Gram-negative bacteria produce β-lactamases which makes them more resistant to penicillins. The released β-lactamase cannot pass through the outer membrane so are trapped in the periplasmic space between the cell membrane and outer membrane. As a result, any penicillin penetrates the outer membrane encounters a higher concentration of β-lactamase than it would with Gram-positive bacteria.

3- High levels of transpeptidase enzyme (PBPs) produced

In some Gram-negative bacteria, excess quantities of transpeptidase are produced and penicillin is incapable of inactivating all the enzyme molecules present.

4- Affinity of the transpeptidase enzyme (PBPs) to penicillin

Several forms of the transpeptidase enzyme are present within any bacterial cell and has different affinity for the different β-lactams. For example, early strains of *S. aureus* contained transpeptidase enzymes having high affinity for penicillin so inhibited effectively.

Penicillin-resistant strains of *S. aureus* acquired a transpeptidase enzyme called PBP2a which has a much lower affinity to penicillins. The presence of low-affinity transpeptidases is also a problem with enterococci and pneumococci.

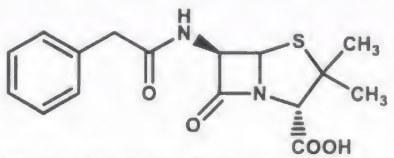
5- Transport back (efflux pump) across the outer membrane of Gram-negative bacteria

Some Gram-negative bacteria have proteins in the outer membrane which capable of pumping penicillin out (efflux) of the periplasmic space, thus lowering its concentration and effectiveness.

6- PBPs Mutations and genetic transfers

Mutations can occur which will affect any or all of the above mechanisms such that they are more effective in resisting the effects of β-lactams. Small portions of DNA carrying the genes required for resistance can also be transferred from one cell to another by means of genetic vehicles called plasmids.

Properties & drawbacks of Penicillin G



Benzylpenicillin (Penicillin G)

Benzylpenicillin (penicillin G; Pen G) is

- Active against a range of bacterial infections.
- Non-toxic, penicillins are amongst the safest drugs.
- Lacks serious side effects for most patients.

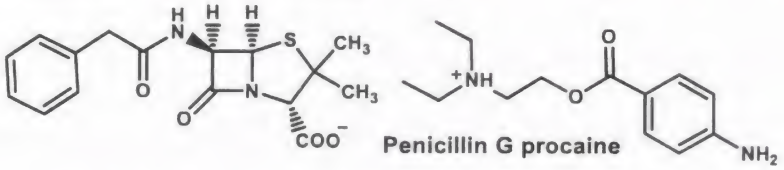
However, there are various drawbacks.

- Short duration.
- Causes allergic reactions in some individuals.

- Acid sensitivity (cannot be taken orally because it is broken down by stomach acids).
- β-Lactamase sensitivity.
- Limited or a narrow spectrum of activity (mainly against Gram-positive bacteria).

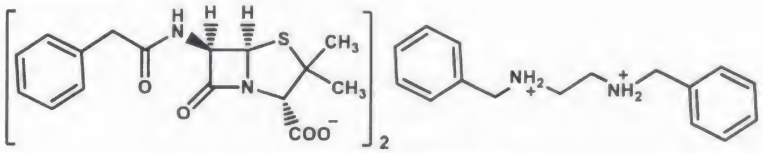
Penicillins with long duration

Penicillin G procaine (procaine penicillin):



- ✓ It is a combination of penicillin G and procaine HCl.
- ✓ It is slowly absorbed into the circuiation after IM injection and hydrolyzed to benzylpenicillin.
- ✓ It is used where prolonged low concentrations of benzylpenicillin are required.

Penicillin G Benzathine:

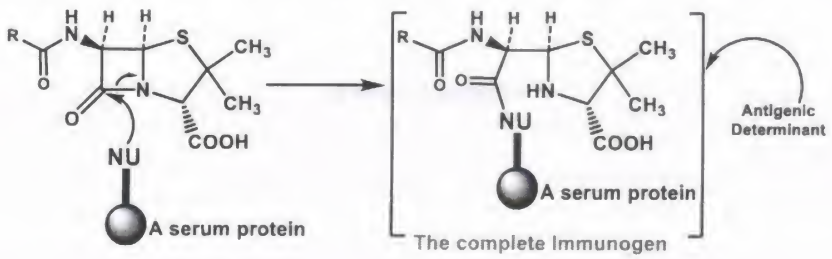


- ✓ It is very water insoluble, so injected IM, where benzathine slowly releases the penicillin making the combination long acting (2-4 weeks after single IM dose).
- ✓ It is also used to prevent rheumatic fever.

Allergic Reactions

Penicillins cause allergic reactions in some individuals, varying from rash to immediate anaphylactic shock, Why??

Penicillins molecules can react with nucleophilic groups on body proteins, the β-lactam ring is opened and the penicillin is covalently linked to the protein.

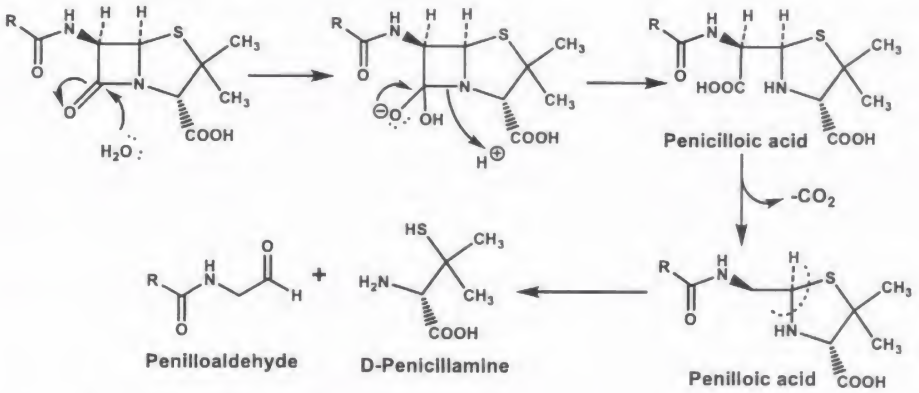


Acid sensitivity of penicillins.

There are three reasons for the acid sensitivity of penicillin G.

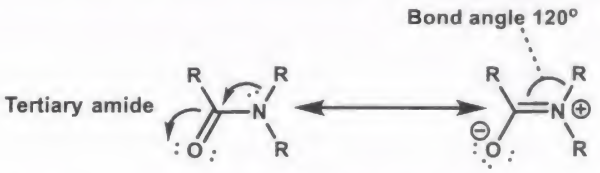
1- Ring strain:

- ✓ The bicyclic system in penicillin consists of a four-membered ring fused to a five-membered ring. As a result, penicillin suffers large angle and torsional strains.
- ✓ Acid-catalyzed ring-opening relieves these strains by breaking open the more highly strained β-lactam ring.

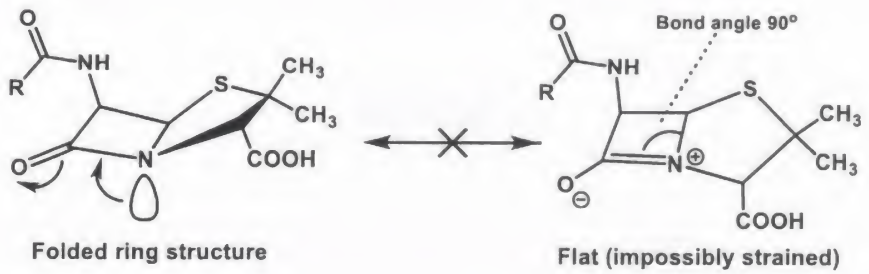


2- A highly reactive β-lactam carbonyl group

- ✓ The carbonyl group in the β-lactam ring is highly susceptible to nucleophiles and does not behave like a normal tertiary amide!?
- ✓ 3ry amide is resistant to nucleophilic attack because the carbonyl group is resonance stabilized by the neighboring nitrogen atom. The nitrogen can feed its lone pair of electrons into the carbonyl group to form a dipolar structure with bond angles of 120°.

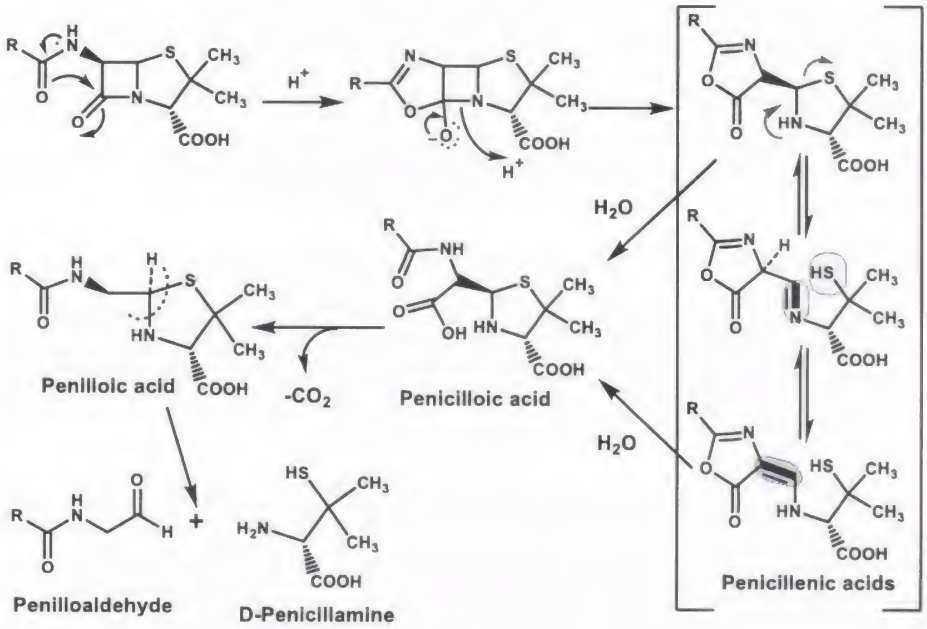


- ✓ This resonance stabilization is impossible for the β-lactam ring because of the increase in angle strain that would result in having a double bond within a four-membered β-lactam ring.



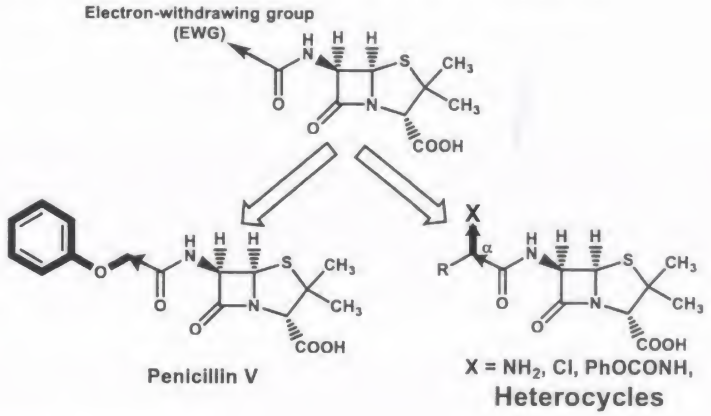
3- Influence of the acyl side chain (neighboring group participation):

Penicillin G has a self-destruct mechanism built into its structure.



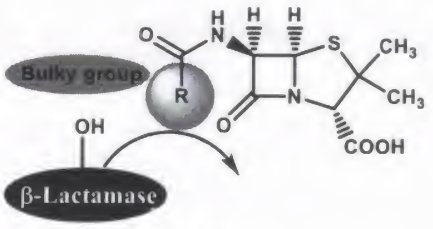
Acid-Resistant (Stable) Penicillins

- ✓ Inserting an electron-withdrawing group (EWG) in the acyl side will decreases the electron density on the side-chain carbonyl and reduce its tendency to act as a nucleophile thus protects penicillins from acid degradation.

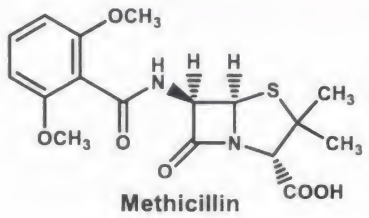


β-Lactamase-resistant penicillins

- ✓ The strategy of steric shields was used successfully to block penicillin from accessing the penicillinase or β-lactamase active site by placing a bulky group on the side chain.



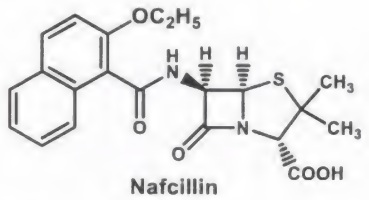
❋ **Methicillin:**



Methicillin

✓ In methicillin, Steric shields are two O–OCH_3 on the aromatic ring.

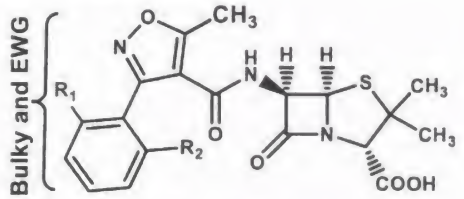
❋ **Nafcillin**



Nafcillin

✓ Substitution at the 2-position of a 1-naphthyl system by ethoxy group increases the steric hindrance of the acyl group and confer more β-lactamase resistance.

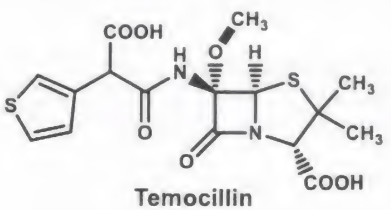
❋ **The isoxazolyl penicillins**



Oxacillin $R_1 = R_2 = H$
Cloxacillin $R_1 = Cl$, $R_2 = H$
Flucloxacillin $R_1 = Cl$, $R_2 = F$
Dicloxacillin $R_1 = Cl$, $R_2 = Cl$

✓ The isoxazoyl penicillins, particularly those with an electronegative substituent in the 3-phenyl group are also resistant to acid-catalyzed hydrolysis of the β-lactam (i.e. orally active).

❋ **Temocillin**



Temocillin

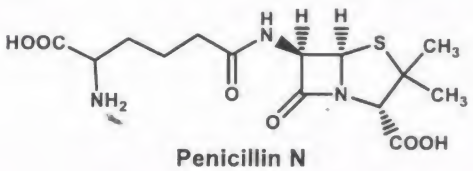
✓ Temocillin is a β-lactamase-resistant carboxypenicillin with 6α-OCH_3 group.

✓ It is reserved for the treatment of infections caused by β-lactamase producing strains of Gram-negative bacteria, including those resistant to third generation cephalosporins.

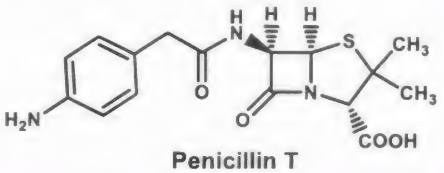
Broad-spectrum penicillins

The variations in the side chain and gave the following results:

- ***Hydrophobic (lipophilic) groups*** on the side chain favor activity against Gram-positive bacteria, but result in poor activity against Gram-negative bacteria.
- If the hydrophobic character is increased, there is little effect on Gram-positive activity, but activity drops even further against Gram-negative bacteria.
- ***Hydrophilic groups*** on the side chain have little effect on Gram-positive activity (e.g. penicillin T) or cause a reduction of activity (e.g. penicillin N), however, they lead to an increase in activity against Gram-negative bacteria.



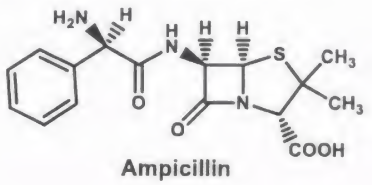
Penicillin N



Penicillin T

- ✓ Enhancement of Gram-negative activity is found to be greatest if the hydrophilic group (e.g. NH_2, OH, COOH) is attached to the carbon that is α to the carbonyl group on the side chain, why?
- ✓ There are three classes of broad-spectrum antibiotics, all of which have an α-hydrophilic group: -
  - The aminopenicillins.
  - The carboxypenicillins.
  - The Ureidopenicillins.

A- The α-aminopenicillins

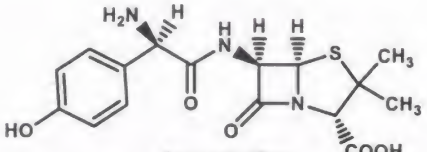


Ampicillin

- ✓ Ampicillin is acid stable.
- ✓ Sensitive to β-lactamase enzyme.
- ✓ Only 40% of the oral dose was absorbed, Why?
- ✓ The problem of poor absorption through the gut wall is due to the dipolar nature of the molecule since it has both free NH_2 and COOH groups which are amphoteric and forms Zwitterr ions (difficult solubility in both acid and alkaline media).

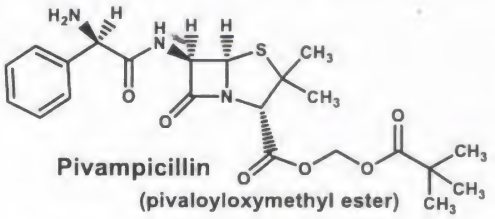
This problem can be solved by:

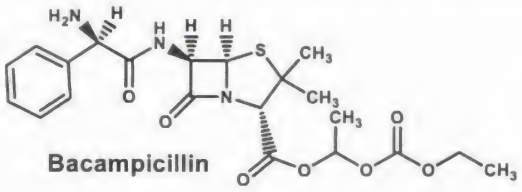
1- Shifting the isoelectric point to the acid side by introducing para-phenolic OH group into the side-chain phenyl → **Amoxicillin**: - 80% of the dose is absorbed after oral administration.



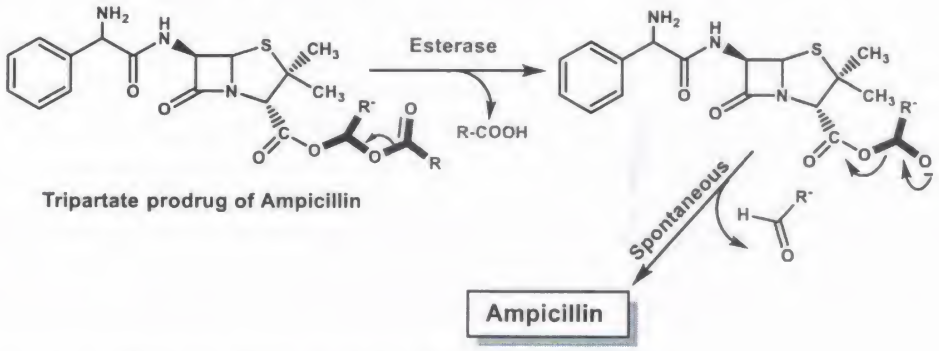
2- Using a prodrug where one of the polar groups is masked with a protecting group. This group is removed metabolically once the prodrug has been absorbed.

✓ 'Eextended or double' esters (Acyloxymethyl esters) contain a second ester group further away from the penicillin nucleus, so is more exposed to attack by esterases.

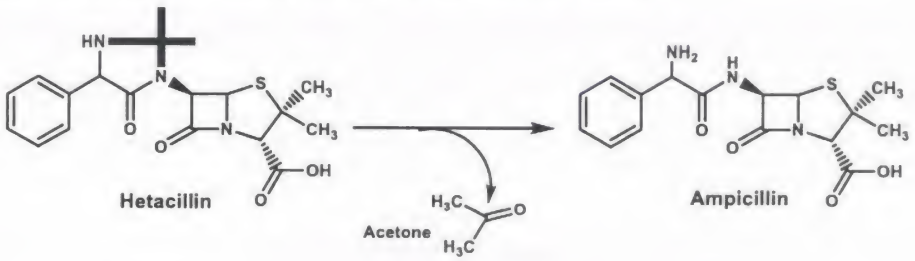




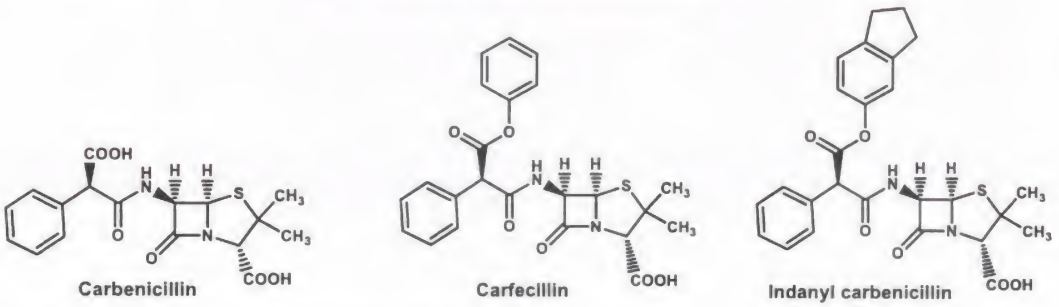
✓ Hydrolysis of the terminal ester gives an unstable hydroxymethyl ester, which spontaneously decomposes to ampicillin.



3- Hetacillin is a Mannich base prodrug of ampicillin which:

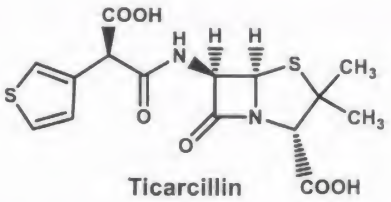


B- The α-carboxypenicillins



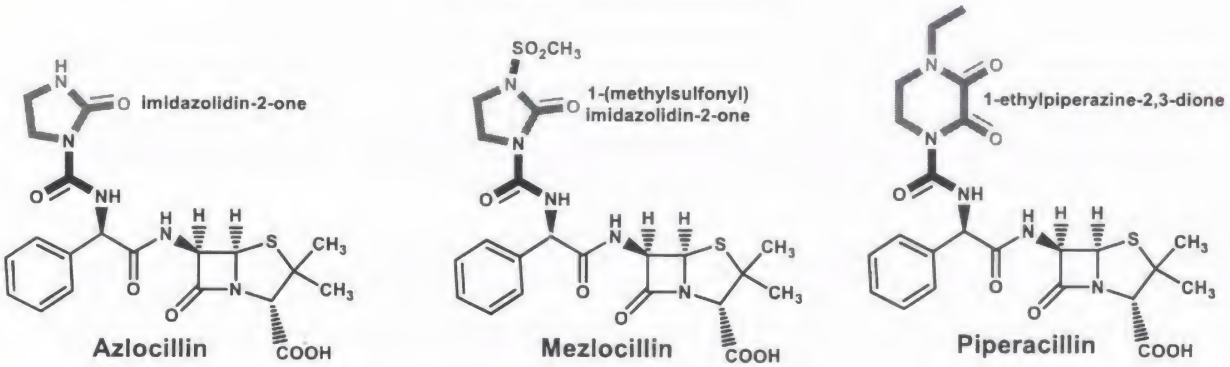
- ✓ Carbenicillin* shows a broad spectrum of activity.
- ✓ The drug is sensitive to β-lactamases and is acid unstable.
- ✓ **Carfecillin** and **Indanyl carbenicillin** are prodrugs for carbenicillin and show an improved absorption through the gut wall.

Ticarcillin



- ✓ Ticarcillin is a sulfur-based bioisostere of carbenicillin that cannot decarboxylate as carbenicillin does.
- ✓ Like carbenicillin, is unstable in acid and, therefore, must be administered parenterally.
- ✓ It has fewer side effects.

C- The ureidopenicillins



- ✓ They have a urea functional group at the α-position.
- ✓ They have better properties and activity than carboxypenicillins.
- ✓ They are generally more active against Gram-negative bacteria.
- ✓ β-lactamase sensitive and are unstable under acidic conditions.
- ✓ Tazobactam is often co-administered with piperacillin.

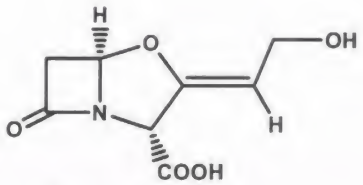
β-Lactamase Inhibitors

- ✓ They are β-lactam agents that show some weak antibacterial activity, but have higher capacity to inactivate β-lactamase.
  - They are often given in combination with penicillins.
  - They often referred as Sentry drugs.

Mechanism of action

- ✓ Inhibit the β-lactamase enzyme by irreversible binding to the enzyme and allows the penicillins molecules to attack the peptidoglycan cell wall in order to destroy the bacterial cell. So generally termed as suicidal substrates.

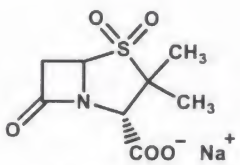
Clavulanic acid



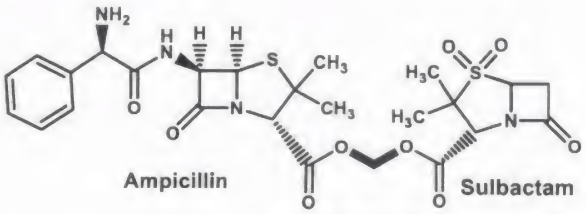
Clavulanic acid

- ✓ It is an oxapenam derivative.
- ✓ It has weak and unimportant antibiotic activity, but it is a powerful and irreversible inhibitor of most β-lactamases.
- ✓ It is used as a sentry drug in combination with amoxicillin (Augmentin)

Sulbactam



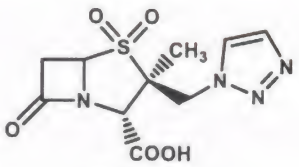
Sulbactam



Sultamicillin

- ✓ It is a semisynthetic Penicillanic acid sulphone derivatives.
- ✓ It has a broader spectrum of activity against β-lactamases than clavulanic acid.
- ✓ It is combined with ampicillin for **IV** administration in a preparation called Unasyn.
- ✓ **Sultamicillin** is a ***double ester of formaldehyde hydrate*** in which one of the hydroxyl groups has been esterified with ampicillin and the other with sulbactam.

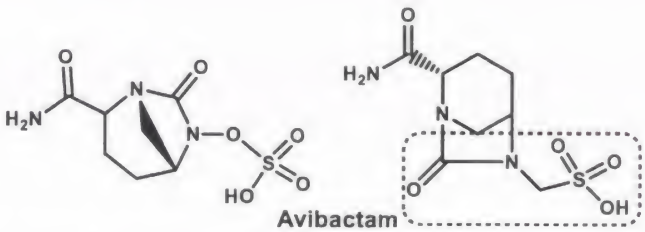
Tazobactam



Tazobactam

- ✓ Tazobactam is a Penicillanic acid sulphone derivatives.
  - ✓ It is combined with ***Piperacillin and Ceftolozane***.

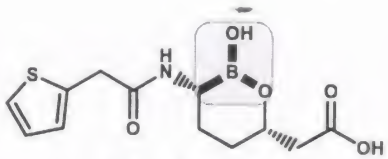
Avibactam



Avibactam

- ✓ Approved by the FDA on 2015 to be used in combination with ceftazidime for treating complicated urinary tract (cUTI) and intra-abdominal infections (cIAI).

Vaborbactam



Vaborbactam

- ✓ Approved by the FDA on August 2017 to be used in combination with meropenem for treating complicated urinary tract infection (cUTI).